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Signed Andrew George

Dated 19 July 2000

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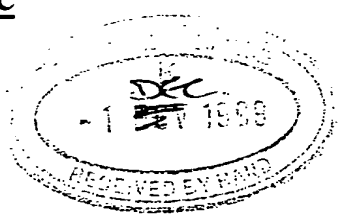
1/77

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)



The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your Reference — GL/PG3839

2. Patent application number *(The Patent office will fill in this part)* — **9928437.4** — 01 DEC 1999

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

GLAXO GROUP LIMITED
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Patents ADP number *(if you know it)* 07317870002

If the applicant is a corporate body, give the country/state of its corporation

4 Title of the invention — **MEDICAL USES**

5 Name of your agent *(if you know one)* — **GRAHAM LANE**
(SEE CONTINUATION SHEET)

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

GLAXO WELLCOME PLC
GLAXO WELLCOME HOUSE, BERKELEY AVENUE
GREENFORD, MIDDLESEX
UB6 ONN, GB

Patents ADP number *(if you know it)*

6. If you are declaring priority from one or more earlier patent applications, give the country and date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of Filing (day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing (day / month / year)

8. Is a statement of inventorship and of right to grant a patent required in support of this request? *(Answer yes if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.

YES

See note (d))

9. Enter the number of sheets for any of the

Patents Form 1/77

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Continuation sheets of this form	1
Description	15
Claim(s)	1
Abstract	1
Drawing(s)	-

10. If you are also filing any of the following, state how many against each item

Priority Documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patent Form 9/77)*	
Request for substantive examination *(Patent Form 10/77)*	
Any other documents *(please specify)*	

11. **I/We request the grant of a patent on the basis of this application**

Signature **GRAHAM LANE** **Date 1 December 1999**
AGENT FOR THE APPLICANTS

12. Name and daytime telephone number of person to contact in the United Kingdom

Kim Allen

0181-966 5721

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(See Page 1 No. 5)

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MEDICAL USES

The present invention relates to new uses for EP4 receptor antagonists.

The EP4 receptor is a 7-transmembrane receptor and its natural ligand is the prostaglandin PGE_2. PGE_2 also has affinity for the other EP receptors (types EP1, EP2 and EP3).

Compounds exhibiting EP4 binding activity have been described in WO98/55468 and EP0855389. GB2330307 describes the use of EP4 antagonists in the treatment of conditions with accelerated bone resorption.

It has now been found that EP4 receptor antagonists are of use in the treatment of migraine, neuropathic pain, colon cancer and in increasing the latency of HIV infection.

It is believed that selective EP4 receptor antagonists exhibit a number of advantages over current non-steroidal anti-inflammatory (NSAID) and cyclo-oxygenase-2 inhibitor (COX-2i) drugs which act via a number of prostaglandin pathways. By selectively inhibiting the EP4 receptor, the beneficial activities of other prostaglandin pathways are retained. The use according to the instant invention therefore provides greater efficacy and improved gastro-intestinal safety over NSAIDs.

The present invention provides the novel use of an EP4 receptor antagonist in the manufacture of a medicament for use in the treatment of migraine, neuropathic pain, colon cancer, and for increasing the latency of HIV infection.

In a further aspect the invention provides a novel method of increasing the latency of HIV infection; and for treating migraine, neuropathic pain, and colon cancer; in a mammal, including man, comprising administration of an effective amount of an EP4 receptor antagonist.

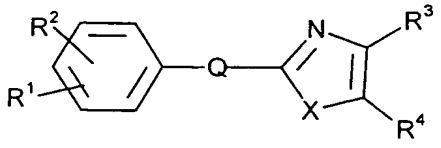
It is to be understood that reference to treatment as used herein includes treatment of established symptoms and prophylactic treatment, unless explicitly stated otherwise.

Suitable EP4 receptor antagonsits for use in the present invention include those described in GB2330307, WO98/55468 and EP0855389 incorporated by reference herein. A preferred EP4 receptor antagonist for use in the present invention is the compound [4-(4,9-diethoxy-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetic acid and pharmaceutically acceptable derivatives thereof of formula (IB) below.

Compounds described in GB2330307 are [1α(Z),2β,5α]-(±)-7-[5-[[(1,1'-Biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid and the physiologically acceptable salts and solvates thereof and [1R[1α(Z),2β,5α]]-(-)-7-[5-[[1,1'-Biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid and the physiologically acceptable salts and solvates thereof which are selective antagonists at the EP4 receptor.

[1α(Z),2β,5α]-(±)-7-[5-[[(1,1'-Biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid and the physiologically acceptable salts and solvates thereof and [1R[1α(Z),2β,5α]]-(-)-7-[5-[[1,1'-Biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid and the physiologically acceptable salts and solvates thereof may be prepared and formulated according to the methods described in UK Patent Application No GB 2075503.

Compounds described in WO98/55468 are azole compounds of formula (I):



(I)

wherein R^1 is lower alkyl substituted with hydroxy, protected carboxy or carboxy; carboxy; protected carboxy; carbamoyl; a heterocyclic group; cyano; hydroxy; halo(lower)alkylsulfonyloxy; lower alkoxy optionally substituted with hydroxy or carbamoyl; aryl substituted with carboxy, protected carboxy, carbamoyl or a

heterocyclic group; or amino optionally substituted with protected carboxy or lower alkylsulfonyl,

R^2 is hydrogen or lower alkyl,

R^3 is aryl optionally substituted with halogen,

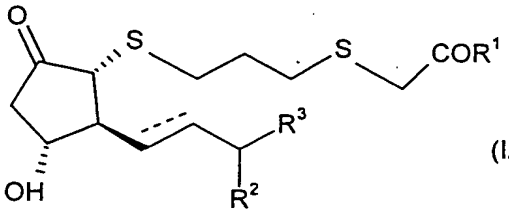
R^4 is aryl optionally substituted with halogen,

Q is $-A^1-\overset{A^2}{\bigcirc}-A^3-$ [in which $-A^1-$ is a single bond or lower alkylene,

$\overset{A^2}{\bigcirc}$ is cyclo $(C_5\text{-}C_9)$alkene, cyclo $(C_3\text{-}C_9)$alkane, bicylco$(C_6\text{-}C_9)$alkene or bicyclo $(C_5\text{-}C_9)$alkane, and $-A^3-$ is a single bond lor lower alkylene], and

X is O, NH or S; which may be prepared according to the methods described therein.

Compounds described in EP0855389 are 3,7-dithiaprostanoic acid derivatives of the formula (IA):



(IA)

(wherein R^1 is hydroxy, C1-4alkoxy or a group of the formula:

$-NR^6R^7$

wherein R^6 and R^7, independently, are hydrogen atom or C1-4alkyl,

R^2 is hydrogen atom or hydroxy,

R^3 is

(i) C1-8alkyl, C2-8alkenyl or C2-8alkynyl,

(ii) phenyl or C3-7cycloalkyl,

(iii) C1-8alkyl, C2-8alkenyl or C2-8alkynyl substituted by phenyl or C3-7 cycloalkyl,

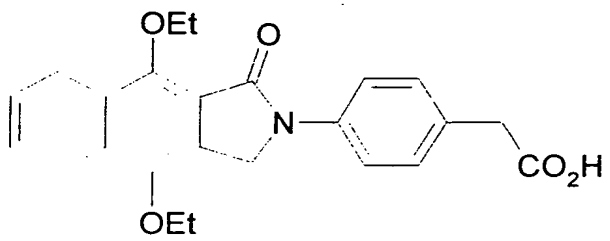
with the provisio that alkyl, alkenyl, alkynyl in (i) or (iii) may be substituted by one hydroxy group, when R^2 is hydrogen atom;

the symbol ---- is a double or single bond;

the formula including the 8-epi equilibrium compound thereof);

a non-toxic salt thereof or a cyclodextrin clathrate thereof, which may be prepared according to the methods described therein.

As mentioned above, a preferred EP4 receptor antagonist for use in the present invention is the compound [4-(4,9-diethoxy-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetic acid of formula (IB) below.



(IB)

The compound of formula (IB) and pharmaceutically acceptable derivatives thereof is novel and therefore forms a further feature of the invention.

The ability of the compounds to inhibit EP4 receptors may be demonstrated in the Human EP_4 Scintillation Proximity Assay.

Quantification of radioligand binding by scintillation proximity assay (SPA) is a long-established principle. Briefly, the affinity of compounds for a receptor is assessed by the specific competition between known quantities of radiolabelled ligand and compound for that receptor. Increasing concentrations of compound reduce the amount of radiolabel that binds to the receptor. This gives rise to a diminishing scintillation signal from SPA beads coated with membranes that bear the receptor. The signal may be detected with a suitable scintillation counter and the data generated may be analysed with suitable curve-fitting software.

The human EP_4 SPA assay (hereafter referred to as 'the assay') utilises membranes prepared from Baby Hamster Kidney cells (BHK cells) infected with Semliki Forest Virus (SFV). The virus is previously transfected with an SFV-1 RNA construct containing the hEP_4 receptor. Cells washed free of media are homogenised in a pH-buffered medium containing peptidase inhibitors. A

suitable buffer is of the following composition: 50mM HEPES, 1mM EDTA, 25μg/ml bacitracin, 100μM leupeptin, 1mM PMSF, 2μM Pepstatin A, pH adjusted to 7.4 with KOH. Following removal of cell debris by a low-speed centrifugation, a pellet of membranes is prepared by a high-speed (48000g) centrifugation of the resulting supernatant. Membrane suspensions such as that described may be stored at -80°C until used.

For assay, membranes expressing human EP_4 receptors are diluted in a pH-buffered medium and mixed with SPA beads coated with a suitable substance to facilitate the adhesion of membranes to the beads. The concentrations of membrane protein and SPA beads chosen should result in SPA binding signal of at least 300 corrected counts per minute (CCPM) when tritiated radioligand at a concentration close to its K_d (affinity value) is combined with the mixture. Non-specific binding (nsb) may be determined by competition between the radiolabelled ligand and a saturating concentration of unlabelled ligand. In order to quantify the affinity of EP4 receptor antagonists, compounds are diluted in a stepwise manner across the wells of a 96-well plate. Radioligand, compound, and unlabelled ligand are then added to a 96-well plate suitable for the measurement of SPA binding signals prior to the addition of bead / membrane mixture to initiate the binding reaction. Equilibrium may be achieved by incubation at room temperature for 120 minutes prior to scintillation counting. The data so generated may be analysed by means of a computerised curve-fitting routine in order to quantify the concentration of compound that displaces 50% of the specific radioligand binding (IC_{50}). The affinity (pK_i) of the compound may be calculated from the IC_{50} by application of the Cheng-Prusoff correction. Suitable reagents and protocols are: reaction buffer containing 50mM HEPES, 10mM $MgCl_2$, pH adjusted to 7.4 with KOH; SPA beads coated with wheatgerm agglutinin; 1.25nM [^{3}H]-prostaglandin E_2 as radioligand; 10μM prostaglandin E_2 as unlabelled ligand; a three-fold dilution series of compound starting at 10μM and ending at 0.3nM is adequate.

By application of this technique, 4-(4,9-diethoxy-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetic had a pKi of 7.00 $\pm$ 0.28 (mean $\pm$ standard deviation of the mean; n = 87).

The novel use of EP4 receptor antagonists in the treatment of neuropathic pain has been demonstrated in the following test.

The chronic constriction injury (CCI) model was used to induce the neuropathic hypersensitivity (Bennett & Xie, 1988) in male random hooded rats.

Under isoflurane anaesthesia, the common left sciatic nerve was exposed at mid thigh level and four loose ligatures of Chromic gut tied around it. The wound was then closed and secured using suture clips. The surgical procedure was identical for the sham operated animals except the sciatic nerve was not ligated. The rats were allowed a period of seven days to recover from the surgery before behavioural testing began.

4-(4,9-diethoxy-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetic acid (10mgkg-1 b.i.d. PO) was dosed chronically for 14 days (days 20-33 post-operative). A reversal of the CCI-induced decrease in paw withdrawal threshold became apparent following 3 days of chronic dosing which was maximal after 1 week. This reversal was maintained throughout the remainder of the dosing period. Following cessation of the drug treatment the paw withdrawal threshold returned to that of the vehicle treated CCI-operated animals.

The compounds for use in the invention may be administered orally at a dose of from 0.1 to 10 mg/kg body weight per day and more particularly 0.3 to 3 mg/kg body weight per day, calculated as the free base. The dose range for adult human beings is generally from 8 to 1000 mg/day, such as from 35 to 800 mg/day, preferably 20 to 200 mg/day, calculated as the free base.

The precise amount of the compounds administered to a host, particularly a human patient, will be the responsibility of the attendant physician. However, the dose employed will depend upon a number of factors including the age and sex of the patient, the precise condition being treated and its severity, and the route of administration.

The compounds and their pharmaceutically acceptable derivatives are conveniently administered in the form of pharmaceutical compositions. Such

compositions may conveniently be presented for use in conventional manner in admixture with one or more physiologically acceptable carriers or excipients.

While it is possible for the compounds to be administered as the raw chemical, it is preferable to present it as a pharmaceutical formulation. The formulations comprise the compounds together with one or more acceptable carriers or diluents therefor and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for oral, parenteral (including subcutaneous e.g. by injection or by depot tablet, intradermal, intrathecal, intramuscular e.g. by depot and intravenous), rectal and topical (including dermal, buccal and sublingual) administration although the most suitable route may depend upon for example the condition and disorder of the recipient. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the compounds ("active ingredient") with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

Formulations suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets (e.g. chewable tablets in particular for paediatric administration) each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating,

surface active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of a sterile liquid carrier, for example, water-for-injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter, hard fat or polyethylene glycol.

Formulations for topical administration in the mouth, for example buccally or sublingually, include lozenges comprising the active ingredient in a flavoured basis such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a basis such as gelatin and glycerin or sucrose and acacia.

The compounds may also be formulated as depot preparations. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

In addition to the ingredients particularly mentioned above, the formulations may include other agents conventional in the art having regard to the type of

formulation in question, for example those suitable for oral administration may include flavouring agents.

The EP4 receptor antagonist compounds for use in the instant invention may be used in combination with other therapeutic agents, for example COX-2 inhibitors, such as celecoxib, rofecoxib, valdecoxib or parecoxib; 5-lipoxygenase inhibitors; low dose aspirin; NSAID's, such as diclofenac, indomethacin or ibuprofen; leukotriene receptor antagonists; DMARD's, for example TNF inhibitors (such as enbril); methotrexate; adenosine 1 agonists; sodium channel antagonists, such as lamotrigene; NMDA antagonists, such as glycine antagonists; and $5HT_1$ agonists, such as triptans, for example sumatriptan, naratriptan, zolmitriptan, eletriptan, frovatriptan, almotriptan or rizatriptan. When the compounds are used in combination with other therapeutic agents, the compounds may be administered either sequentially or simultaneously by any convenient route. The invention thus provides, in a further aspect, the use of a combination comprising an EP4 receptor antagonist with a further therapeutic agent in the treatment of migraine, neuropathic pain, colon cancer and in increasing the latency of HIV infection.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier or excipient comprise a further aspect of the invention. The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

When an EP4 receptor antagonist is used in combination with a second therapeutic agent active against the same disease, the dose of each compound may differ from that when the compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

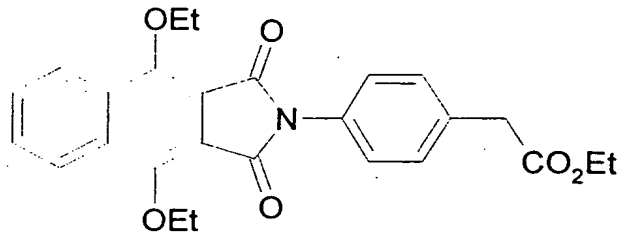
Preferred unit dosage formulations are those containing an effective daily dose, as herein above recited, or an appropriate fraction thereof, of the active ingredient. Conveniently that may be from 5 mg to 1000 mg, such as from 8 mg

to 1000 mg, more conveniently 35 mg to 800 mg, and most conveniently 20 to 200 mg, calculated as the free base.

The compound of formula (IB) and pharmaceutically acceptable derivatives thereof may be prepared by any method known in the art for the preparation of compounds of analogous structure.

A suitable method for the preparation of compound (IB) and pharmaceutically acceptable derivatives thereof is described below and forms a further aspect of the invention.

Compound (IB) may be prepared by reducing the compound



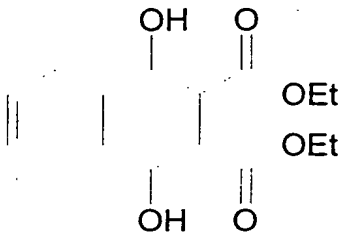
with a suitable reducing agent, for example zinc in acetic acid at elevated temperature, followed by separation of isomers and deprotection (eg. with aqueous base at elevated temperature).

The following Example which should not be construed as constituting a limitation thereto is provided to illustrate the invention.

^{1}H NMR spectra were obtained at 400MHz on a Bruker DPX400 spectrophotometer. J values are given in Hz. Mass spectra were obtained on a Micromass series II MS (electrospray positive or negative

Intermediate 1

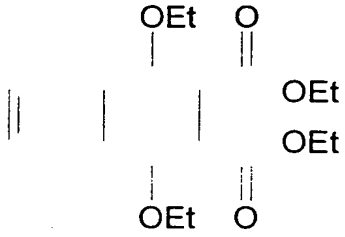
Ethyl 1,4-dihydroxy- 2,3-naphthalenedicarboxylate



Sodium (60g, 2.6mol) was dissolved in ethanol (1.2L) and the mixture was cooled to 40°C. Diethylphthalate (960ml, 4.83mol) was added and the mixture heated under nitrogen until the temperature reached 115°C. Diethyl succinate (211.3g, 1.21mol) was added dropwise over 45 min. The reaction was heated at 115°C for a further 45 min, cooled to room temperature and poured onto water (1.2L). Ethyl acetate (1L) was added and stirred, the layers were separated and the organics were extracted with sodium hydroxide solution (2N, 1L). The combined aqueous was acidified to pH 3 and the mixture extracted with ethyl acetate (2 x 1L). The combined organics were washed with a saturated solution of sodium hydrogen carbonate (2 x 1.5L), then brine, dried ($MgSO_4$), filtered and the solvent evaporated under vacuum. The residue was purified using a 2.5kg Biotage column eluting with 5% ethyl acetate / hexane to give ethyl 1,4-dihydroxy- 2,3-naphthalenedicarboxylate as a white solid, (60g, 16%)
δH $CDCl_3$ 10.44,(2H, s), 8.34,(2H, m), 7.68,(2H, m), 4.37,(4H, q), 1.37,(6H, t).

Intermediate 2

Ethyl 1,4-diethoxy- 2,3-naphthalenedicarboxylate



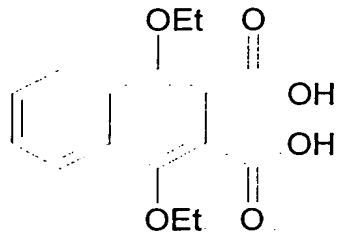
Ethyl 1,4-dihydroxy- 2,3-naphthalenedicarboxylate (30g, 98.6 mmol) and potassium carbonate (150g, 1.09mmol) were stirred in acetone (600ml) under nitrogen. Iodoethane (150g, 0.96mol) was added and the mixture was stirred at reflux overnight. The reaction was cooled, diluted with ethyl acetate and filtered.

The filtrate was evaporated to leave a brown oil, which was dissolved in toluene and washed with potassium hydroxide solution (5%, 150ml) and brine. Drying over magnesium sulphate and evaporation of the solvent gave a yellow solid. Purification using an 800g Biotage column gave ethyl 1,4-diethoxy- 2,3-naphthalenedicarboxylate as a white solid (32g, 90%).

δH $CDCl_3$ 8.16,(2H, m), 7.60,(2H, m), 4.40,(4H, q), 4.18,(4H, q), 1.50,(6H, t), 1.40,(6H, t).

Intermediate 3

1,4-Diethoxy- 2,3-naphthalenedicarboxylic acid

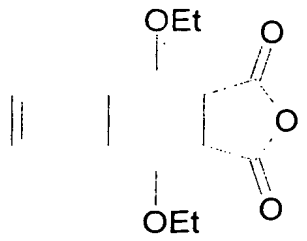


Ethyl 1,4-diethoxy- 2,3-naphthalenedicarboxylate (32g, 89mmol) was added to a solution of sodium hydroxide (20g) in ethanol (200ml) and water (40ml) and stirred for 1.5h at 60°C. The reaction was cooled and the thick white suspension was filtered. The solid was dissolved in a mixture of ethyl acetate (200ml) and water (800ml). The layers were separated and the aqueous was acidified with hydrochloric acid (2M, 120ml). The aqueous was extracted with ethyl acetate (2x) and the combined organics were dried ($MgSO_4$). Evaporation of the solvent under vacuum gave 1,4-diethoxy- 2,3-naphthalenedicarboxylic acid as a white solid (25g, 92%).

δH [$^2H_6$] – DMSO 13.26,(2H, s), 8.15,(2H, m), 7.72,(2H, m), 4.13,(4H, q), 1.42,(6H, t).

Intermediate 4

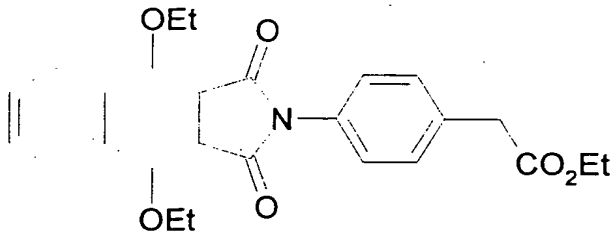
1,4-Diethoxy- 2,3-naphthalenedicarboxylic anhydride



1,4-Diethoxy- 2,3-naphthalenedicarboxylic acid (25g, 82mmol) was added to a solution of thionyl chloride (23.3g) in chloroform (150ml) and stirred at reflux for 1h. The resulting solution was cooled and evaporated to dryness. Further chloroform was added and evaporation repeated to give 1,4-diethoxy- 2,3-naphthalenedicarboxylic anhydride as a yellow solid (23.3g, 99%).
δH [$^2H_6$] – DMSO 8.42,(2H, m), 7.93,(2H, m), 4.53,(4H, q), 1.46,(6H, t).

Intermediate 5

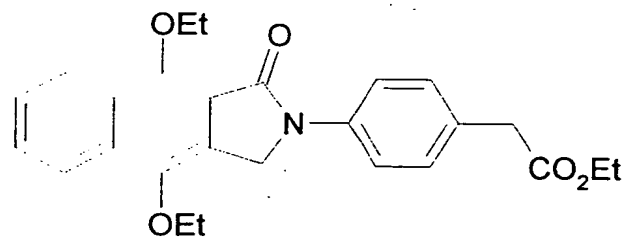
Ethyl[4-(4,9-diethoxy-1,3-dioxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetate



1,4-Diethoxy- 2,3-naphthalenedicarboxylic anhydride (23.3g, 81.5mmol) and ethyl (4-aminophenyl)acetate (14.8g, 82mmol) were refluxed under nitrogen in acetic acid (160ml) overnight. The mixture was cooled to room temperature and poured into water (1L). The white solid was filtered, washed with water and dissolved in dichloromethane (800ml). The solution was washed with water, brine and dried ($MgSO_4$) and the solvent evaporated under vacuum to give ethyl [4-(4,9-diethoxy-1,3-dioxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetate as an off-white solid, 33g, 96%.
δH [$^2H_6$] – DMSO 8.40,(2H, m), 7.87,(2H, m), 7.42,(4H, s), 4.47,(4H, q), 4.12,(2H, q), 3.76,(2H, s), 1.45,(6H, t), 1.21,(3H, t).

Example 1 – Step 1

Ethyl [4-(4,9-diethoxy-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetate

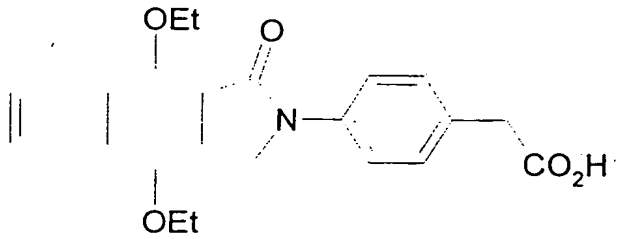


Ethyl [4-(4,9-diethoxy-1,3-dioxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetate (33g, 73mmol) and zinc (90g, 1.38mol) were refluxed in acetic acid for 66h. An additional quantity of zinc (25g, 0.38mol) was added and reflux continued for 18h. The mixture was flitered hot and the filtrate was evaporated to a yellow solid. The solid was purified by 800g Biotage column eluting with 20% ethyl acetate/ hexane to give a white solid, which was triturated in ether to give a white solid. A further fraction was obtained by crystalisation from the ether residues. A total of 10.2g, 32% of ethyl [4-(4,9-diethoxy-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetate was obtained.

δH $CDCl_3$ 8.42,(1H, d), 8.18,(1H, d), 7.88,(2H, d), 7.63,(2H, m), 7.38,(2H, d), 5.00,(2H, s), 4.51,(2H, q), 4.26,(2H, q), 4.18,(2H, q), 3.65,(2H, s), 1.57,(6H, m), 1.28,(3H, t).

Example 1 – Step 2

[4-(4,9-diethoxy-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetic acid



Ethyl [4-(4,9-diethoxy-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetate (5.86g, 13.5mmol) and potassium carbonate (12g) were added to a mixture of

ethanol (146ml) and water (70ml) and heated to reflux for 2h. The solution was cooled to room temperature and the solvent evaporated under vacuum to leave an off-white solid. The solid was slurried in water and the water was evaporated under vacuum. The residue was stirred in hydrochloric acid (2N) for 2h, filtered and washed with water. Drying of the solid at 40° C in a vacuum oven gave [4-(4,9-diethoxy-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetic acid as a white solid (4.5g, 82%)

δH [$^2H_6$] – DMSO 12.27,(1H, b), 8.25,(1H, d), 8.12,(1H, d), 7.86,(2H, d), 7.61,(2H, m), 7.27,(2H, d), 5.10,(2H, s), 4.34,(2H, q), 4.25,(2H, q), 3.54,(2H, s), 1.41,(3H, t), 1.37,(3H, t). MS 406, [MH^+]

The application of which this description and claims forms part may be used as a basis for priority in respect of any subsequent application. The claims of such subsequent application may be directed to any feature or combination of features described herein. The claims may take the form of product, composition, process or use claims and may include, by way of example, one or more of the following claims.

Claims:

1. The use of an EP4 receptor antagonist in the manufacture of a medicament for use in the treatment of migraine, neuropathic pain, colon cancer, and for increasing the latency of HIV infection.

2. A method of increasing the latency of HIV infection; and for treating migraine, neuropathic pain, and colon cancer; in a mammal, including man, comprising administration of an effective amount of an EP4 receptor antagonist.

Abstract

MEDICAL USES

The present invention relates to the use of an EP4 receptor antagonist in the manufacture of a medicament for use in the treatment of migraine, neuropathic pain, colon cancer, and for increasing the latency of HIV infection.